ASSESSMENT OF MECHANICAL AND ANTIBIOTIC ELUTION PROPERTIES OF NOVEL HOLLOW TUBE SURGICAL MESH

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Undergraduate Thesis

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Abstract

Ventral hernias are a major health burden with nearly 350,000 occurrences each year in the United States. A common method of treatment uses a surgical mesh to repair hernias. However, up to 8% of these surgical meshes become infected, and many of them are chronic, antibiotic-resistant infections thought to be caused by biofilms. Preventing the formation of biofilms would alleviate the burden of repeat surgeries due to the infections. This research aims to assess the mechanical and antibiotic elution properties of a novel hollow tube surgical mesh. Relevant properties such as tensile strength, elastic modulus, strain at break, and anisotropy are compared for the hydrated hollow tube mesh and a hydrated mesh similar to the ones that are typically used in hernia repair. The hollow tube mesh is charged with rifampicin in 10% DMSO and evaluated by assay against Staphylococcus aureus. To eliminate residual rifampicin clinging to the exterior of the mesh as the cause for the zone of inhibition, the area of the mesh that came into contact with the rifampicin is removed. Long pieces of rifampicin-charged mesh are tested both sealed and unsealed to ensure that the zones of inhibition are due to elution of the rifampicin through the walls and not leakage out of the ends. The novel hollow tube mesh was shown to be significantly stronger ($p<.002$) and significantly stiffer ($p<10^{-6}$) than the other mesh, but the strain at breaking was not significantly different. The antibiotic elution capabilities of the novel hollow tube mesh were shown not to be due to the solution the rifampicin was in, the loading techniques, the assay procedure, or leakage out of the end of the mesh. The use of a mesh in ventral hernia repair that elutes antibiotics to prevent the formation of biofilms is plausible.

Acknowledgements

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Poster Presentations

Young, M., Peters, C., Dusane, D., Labib, M., Stoodley, P. *Assessment of Mechanical and Antibiotic Elution Properties of Novel Hollow Tube Surgical Mesh.* Poster presented at the 2016 Denman Undergraduate Research Forum at The Ohio State University, Columbus, OH. March 30 2016.

Young, M., Peters, C., Dusane, D., Labib, M., Stoodley, P. *Assessment of Mechanical and Antibiotic Elution Properties of Novel Hollow Tube Surgical Mesh.* Poster presented at the 2016 Center for Microbial Interface Biology Symposium at The Ohio State University, Columbus, OH. March 31-April 1 2016.

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INTRODUCTION

A ventral hernia occurs when the intestines push through the abdominal muscles. This causes nausea, pain and/or pressure at the site of the hernia, and can lead to severe damage to the intestines if left untreated. This is a major health burden because of the estimated 348,000 occurrences each year [1]. Surgery is a common method of treatment for ventral hernias. The surgery typically involves closing the opening in the abdominal muscles either by directly suturing the muscle to itself or by implanting a polypropylene mesh (PPM). Though reported recurrence values vary widely, there is a fair amount of agreement that the use of surgical meshes has less probability of recurrence than other methods of ventral hernia repair. [2,3] A drawback to these surgical procedures is that a significant portion of them become infected at the site of mesh or suture; some studies estimate around 8% result in chronic infection due to biofilms [2,4].

An ideal surgical mesh for hernia repair would be strong enough to remain intact throughout the lifetime of the patient, and it would be a stiffness that does not cause excess stress on the surrounding tissue nor stretch such that the tissue cannot exert sufficient force. It ought to be relatively isotropic, so that its precise orientation does not matter for success in the patient. Ideally, it should also prevent the formation of chronic infection due to biofilms.

A biofilm differs from a regular infection in that it forms on a foreign body's surface, creates a matrix that encapsulates the bacteria, and is resistant to the host's immune cells and traditional antibiotic regimens. Surgical meshes have been shown to be able to support biofilms in vitro, and infections have been shown to be caused by biofilms in some cases [5,6]. Because of the antibiotic resistance properties of biofilms, it would be beneficial to stop colonies of bacteria before they form a biofilm.

This research aims to determine if a novel hollow tube surgical mesh is comparable to an existing commercial surgical mesh in terms of ultimate tensile strength (UTS), stiffness, strain at breaking, and anisotropy of the tensile strength. The research also aims to determine the effectiveness of the hollow

tube surgical mesh in inhibiting the growth of a bacteria known to be found in infected surgical meshes, *S. aureus*, by carrying an antibiotic in its tubes [6].

METHODOLOGY

Mechanical Testing

Mechanical properties such as UTS, and strain at breakage for both the novel hollow tube mesh and the standard mesh were determined in a manner similar to work that has been done on other meshes such that the data is comparable with other results [7]. First, the bulk mesh was cut into a dog-bone shape using a cardboard template. The dog-bone shape, shown in Figure 1A, concentrates the stresses in the center of the sample, away from the clamping area. The testing direction was in the direction that would place the force on the fibers axially, as determined by visual inspection. This direction was denoted the longitudinal direction, and is indicated in Figure 1B. The samples were then soaked in a saline solution for 30 minutes to hydrate the mesh. The width at the narrowest part of the dog bone was measured with calipers and each end of the sample was placed in between two cardboard pieces and clamped tightly. The cardboard allows for a more even force distribution from the clamps. The sample was then pulled with the TESTRESOURCES 100 Family Single Column Electromechanical Universal Test Machine (Figure A2)

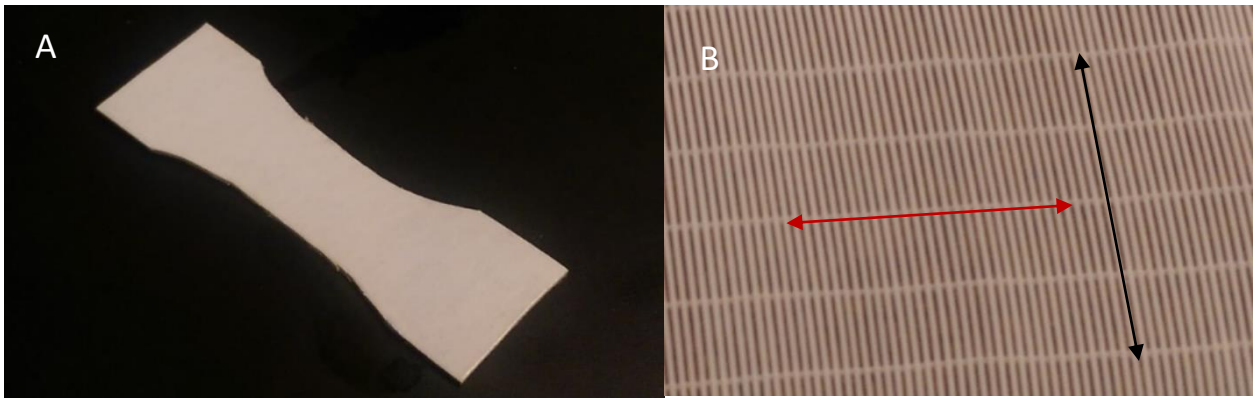

Figure 1: Testing shape and mesh weave. A. Cardboard pattern used to cut the dog bone shape. This shape concentrates the forces in the center region. B. A close up photograph of the novel hollow tube mesh. The longitudinal direction is the direction that places a tensile load on the most fibers, and is indicated by the black arrow. The red arrow indicates the transverse direction, which is perpendicular to the longitudinal direction in the plane of the mesh.

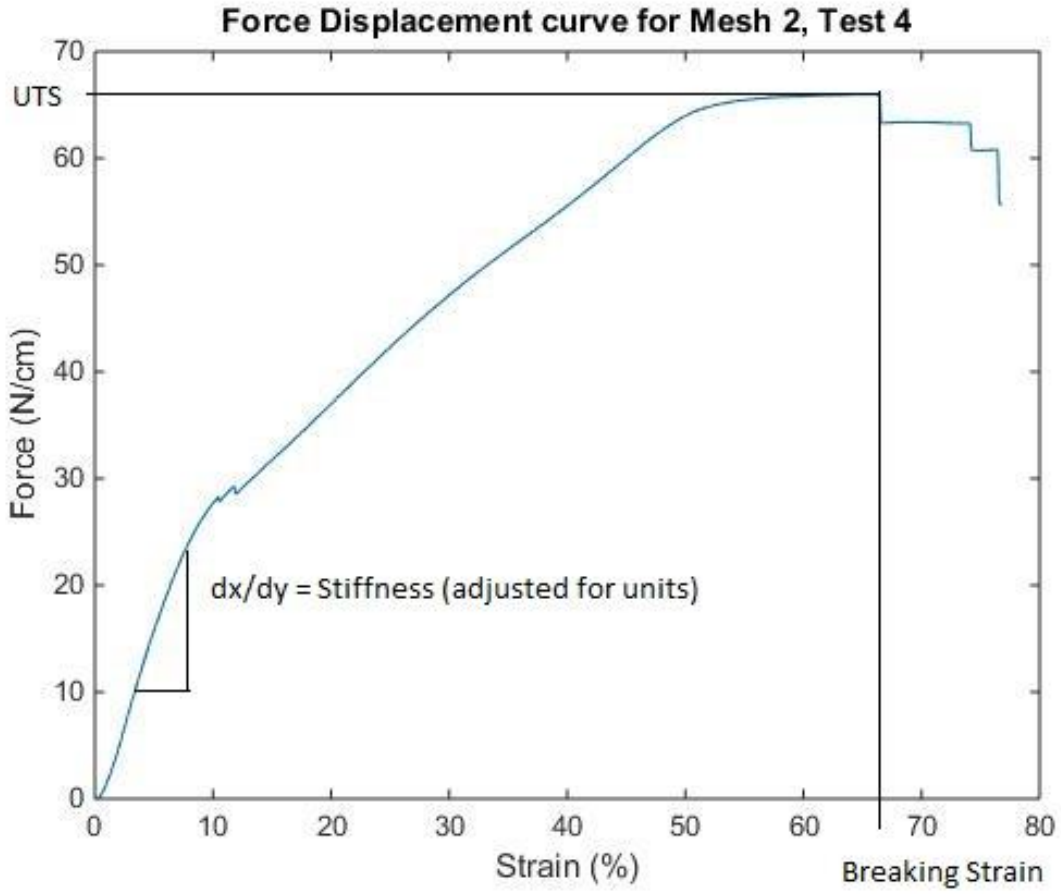

Figure 2: Mechanical testing data collection. An example mechanical test showing how the values that are used were calculated.

at a strain rate of 50 mm/min until the load dropped to 90% of its maximum value. This was considered a sample break. These parameters had been previously used by Pott et al [7]. The UTS (N/cm) was calculated by dividing the maximum force (N) that the sample bared by the measured width (cm). The strain at break was determined by dividing the extension (cm) by the original length, 2.5 cm. An example of how these values were calculated can be seen in Figure 2.

The novel surgical mesh was tested against a commercially available mesh similar to one that might be used in surgery. Typical sample breaks are shown in Figure A3. The UTS, stiffness, and breaking strain data were compared by a two-tailed t-test that assumed unequal variances. The anisotropy of the mesh was determined by comparing the data for tensile strengths in the longitudinal and transverse direction with a two-tailed t-test assuming unequal variances.

Microbiological Assays

Lysogeny broth (LB) agar plates were used to perform the microbiological assays. *S. aureus* (SAP-231) was grown overnight in LB media at 37°C from a frozen stock solution. The plates were inoculated with 100 µL of the SAP-231 suspension, and the suspension was spread evenly across the top face of the LB agar. The solvent and antibiotic tests that were performed without the mesh were done by placing a sterile paper disk on top of the LB agar. The paper disk was then loaded with 10 µL of the appropriate solution. Tests with the mesh were performed by placing a mesh that is loaded with the appropriate solution directly on top of the LB agar. The plate was then incubated for 24 hours at 37°C.

After plate incubation, the plates were imaged. Photographic images were taken with the back facing camera of an HTC One M8. Bioluminescence images were captured with an IVIS in vitro imaging system. The plates were imaged in a dark room, where the number of photons to hit the camera is proportional to the number of live SAP-231 in the plate. The IVIS images are represented by a heat map, where high photon intensity represents a region of more active SAP-231.

Rifampicin Solution

Early attempts at the elution tests used rifampicin dissolved in water. Rifampicin was used because it is not currently used in similar applications, and therefore, a resistance to rifampicin would not be detrimental to other treatments. In clinical applications, other antibiotics would need to be used in combination because rifampicin is notorious for producing mutants. Rifampicin has limited solubility in water, making it difficult to get the rifampicin in sufficient concentrations to be effective for this application. Because of this, 10% DMSO was used as the solvent instead of pure water. Other solvents were not considered.

Mesh Preparation

Early on, the mesh was also loaded by a complex aspiration system. The mesh was rolled up and placed partially inside a silicon tube. The other end of the silicon tubing was attached to a laboratory vacuum system. In order to prevent the mesh from being sucked into the tubing, the tubing was partially clamped around the mesh. This also prevented the negative pressure from being lost by the flow of air around the mesh. The unclamped end of the mesh was dipped into the rifampicin solution, and the solution was drawn into the mesh. The clamped end of the mesh was then fully clamped, sealing the liquid in the mesh. The unclamped end was permanently sealed by melting and pinching the ends of the mesh with a soldering iron. This method was complex, and due to the hydrophobicity of the mesh, was also ineffective. The water solution did not interact favorably with the mesh and it was therefore difficult to trap the solution inside the mesh.

To solve the solubility problems, the rifampicin was dissolved in 10% DMSO instead of water. This had the unintended benefit of also making the interaction between the mesh and the solution much more favorable. Because of the use of 10% DMSO, the mesh could be loaded by the capillary effect. This new loading procedure simplified the process considerably and is shown in Figure A1. The mesh was cut to size, then one end was dipped into the rifampicin in 10% DMSO solution. The capillary effect drew the solution through the tubes. After the liquid reached the top of the tubes, the top was sealed by a soldering iron while the bottom was still in the solution. The seal was checked by touching the seal to a kimwipe. If the kimwipe got wet, the tubes were further sealed. The mesh was removed from the solution, and the part that was dipped into the solution was removed to ensure that any inhibition was not due to rifampicin on the exterior of the mesh. The freshly cut end was sealed with a soldering iron and was ready to be placed on the LB agar plate.

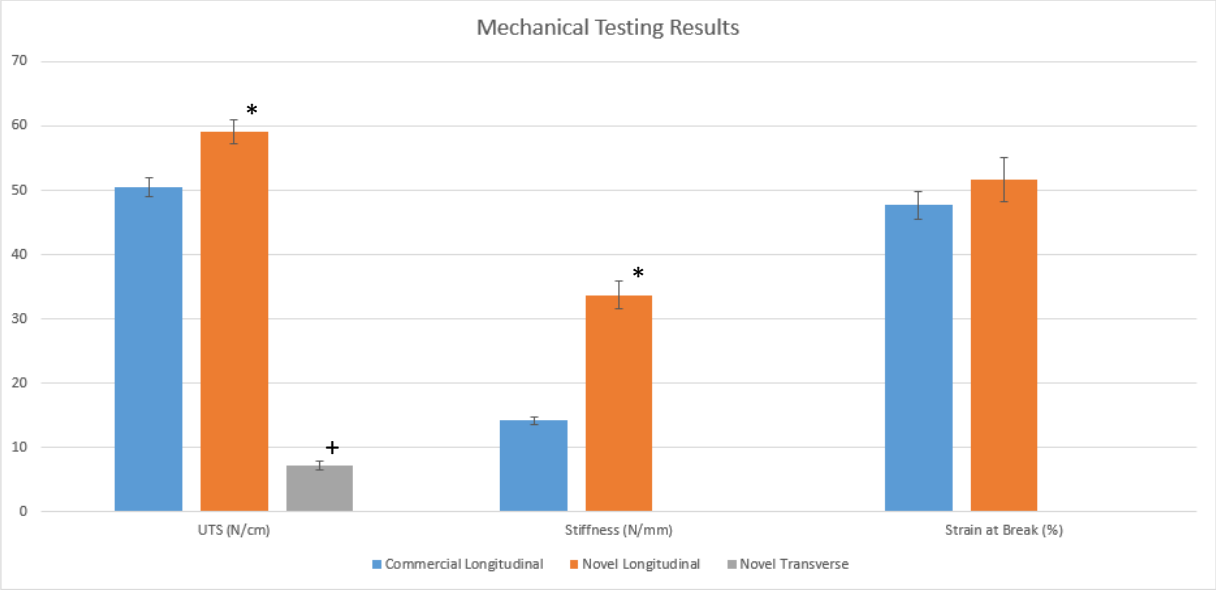

Figure 3: Mechanical testing results. Summary of mechanical data. The novel mesh was significantly stronger and stiffer than the commercial mesh, and the strain at break did not differ significantly. The novel mesh was significantly weaker in the transverse direction. The asterisk means that the mean differs significantly from the commercial mean ($p<.05$). The plus sign means that the mean differs significantly from the longitudinal direction ($p<.05$). The blue bar is the commercial mesh, the orange bar is the novel mesh in the longitudinal direction, and the gray bar is the novel mesh in the transverse direction.

RESULTS

Mechanical Testing

The novel hollow tube mesh had an UTS of 59.09 ± 6.45 N/cm (n = 12) that was significantly stronger ($p < .002$) than the standard mesh with a UTS of 50.46 ± 4.74 N/cm (n = 10) and significantly stiffer than ($p < 10^{-6}$) the standard mesh. The stiffnesses for the novel hollow tube mesh and the standard mesh were 33.65 ± 7.50 N/mm and 14.21 ± 1.85 N/mm, respectively. These data are summarized in Figure 3 and compared to the data found by Pott et al. in Table 1. The strength of the mesh is favorable for use

	This study		From Pott et al.[7]					
	Novel Mesh (Longitudinal)	Commercial Mesh	DYNAMESH_IPOM	PARIETENE	PROLENE	SURGIPRO	ULTRAPRO	VICRYL
UTS (N/cm)	59.09 ± 6.45	50.46 ± 4.74	11.1 ± 6.4	38.9 ± 5.2	84.8 ± 15.0	38.6 ± 12.3	100.9 ± 9.4	78.2 ± 10.5
Breaking Strain (%)	51.62 ± 11.70	47.67 ± 6.75	340 ± 20	294 ± 5	186 ± 7	213 ± 13	195 ± 5	150 ± 6
Stiffness (N/mm)	33.65 ± 7.50	14.21 ± 1.86	0.3 ± 0.1	0.9 ± 0.1	3.6 ± 0.4	1.3 ± 0.3	4.3 ± .4	4.6 ± 0.5

Table 1: Data comparison to Pott et al. Summary of the mechanical properties of the meshes that were tested in this study and the ones that Pott et al. tested. The UTS of the novel mesh in the longitudinal direction is comparable to other meshes. The breaking strain was less than Pott et al. measured, and the stiffness was higher.

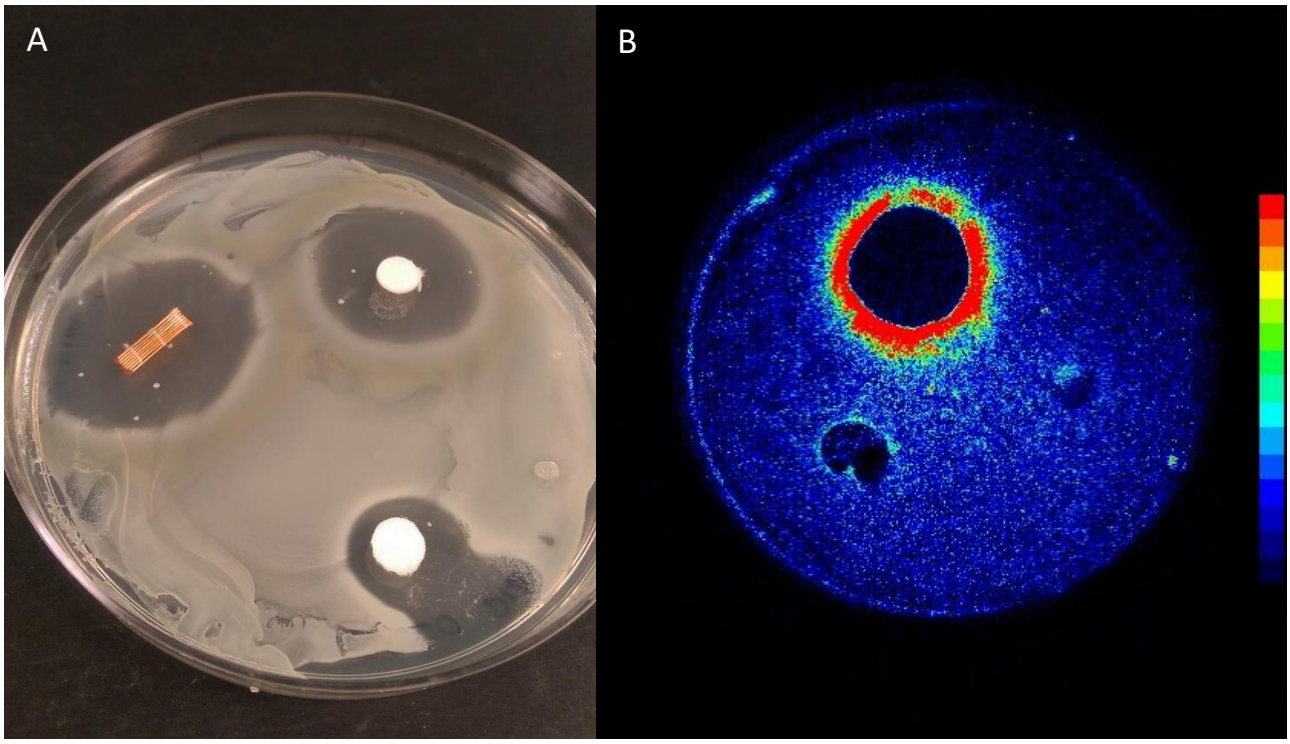

Figure 4: Overall mesh test and primary solvent testing. A. The piece of mesh is charged with the rifampicin solution, and has a zone of inhibition similar to the rifampicin loaded paper disk, which is the top paper disk. The bottom paper disk is charged with only 10% DMSO. It is believed that the zone of inhibition may be due to touching the paper disk with the same tools that touched the rifampicin charged disk and mesh. B. IVIS image of the solvent tests. The large zone of clearance with the red ring around it is a 10% SDS solution that was used as a positive control. The bottom left disk is loaded with 10% DMSO and has little inhibition. The paper disk to the right is an unloaded paper disk. The color scale on the right shows the intensity of the bioluminescence, with red being the most and black being the least.

in applications similar to the ones these are used in. The stiffness, however is much more than those measured by Pott et al. and also much more than the tissue it would be implanted into. [7,8] This may lead to stress concentrations in the native tissue that could cause problems such as fibrosis, which is known to be dependent on mechanical factors. [9]

The anisotropy tests show that the mesh was significantly weaker in the transverse direction with mean UTS in the longitudinal and transverse directions of 59.09 ± 6.45 N/cm and 7.13 ± 1.44 N/cm (n = 4) respectively. These data are also included in Figure 3. This would likely become an issue in vivo, as the mesh will not be loaded uniaxially for the life of the mesh. Being much weaker in one direction may lead to a greater mechanical failure rate, and therefore, a higher rate of recurrence of ventral hernia.

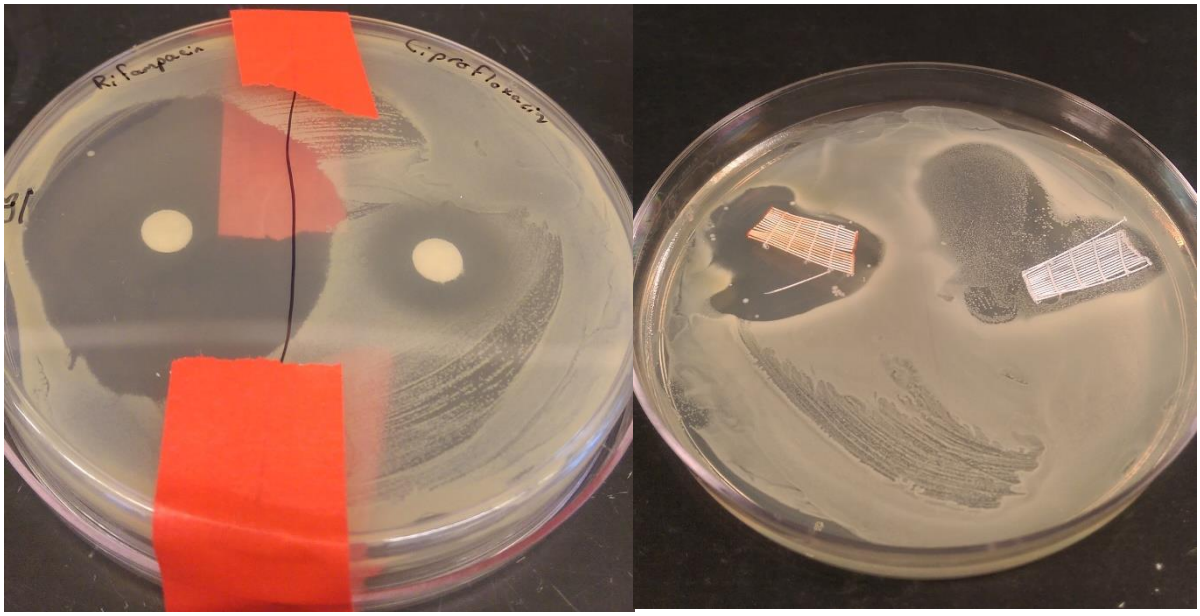

Figure 5: Antibiotic and loaded mesh tests. A. This test shows the test between rifampicin (left) and ciprofloxacin (right). The rifampicin has a larger zone of inhibition, and had a less hazy zone of inhibition. B. Rifampicin charged mesh (left) vs. 10% DMSO charged mesh (right). The DMSO appears to have a large zone of inhibition, but it is much hazier than the rifampicin zone of inhibition. There is an area that looks like it could have connected the two zones, allowing for the rifampicin to come into the DMSO zone, and partially inhibit the SAP-231. This suspect area is highlighted with a red rectangle.

Microbiological Assays

The test of 10 µL rifampicin in 10% DMSO on a paper disk vs 10 µL 10% DMSO on a paper disk showed that the rifampicin increased the zone of inhibition around the disks as shown in Figure 4A. This implies that the rifampicin inhibits the growth of the SAP-231. There is still, however, a zone of inhibition around the 10% DMSO disk. There may have been contamination with the loaded mesh or the disk with the rifampicin solution. The loaded mesh showed a zone of inhibition against the SAP-231. Separate tests of 10% DMSO showed it was inactive against SAP-231, with 10% SDS as a positive control and an unloaded disk as a negative control, as shown in Figure 4B. To test the effectiveness of the rifampicin solution, the solution was run against a solution of ciprofloxacin in water. The rifampicin had a larger zone of clearance and a less hazy zone of clearance, as shown in Figure 5A.

The rifampicin charged mesh created a zone of inhibition around the mesh. This charged mesh was run against a DMSO charged mesh. The DMSO seemed to affect a larger area of bacteria, but the bacteria were not as inhibited inside this zone as evidenced by the cloudiness of the area surrounding the

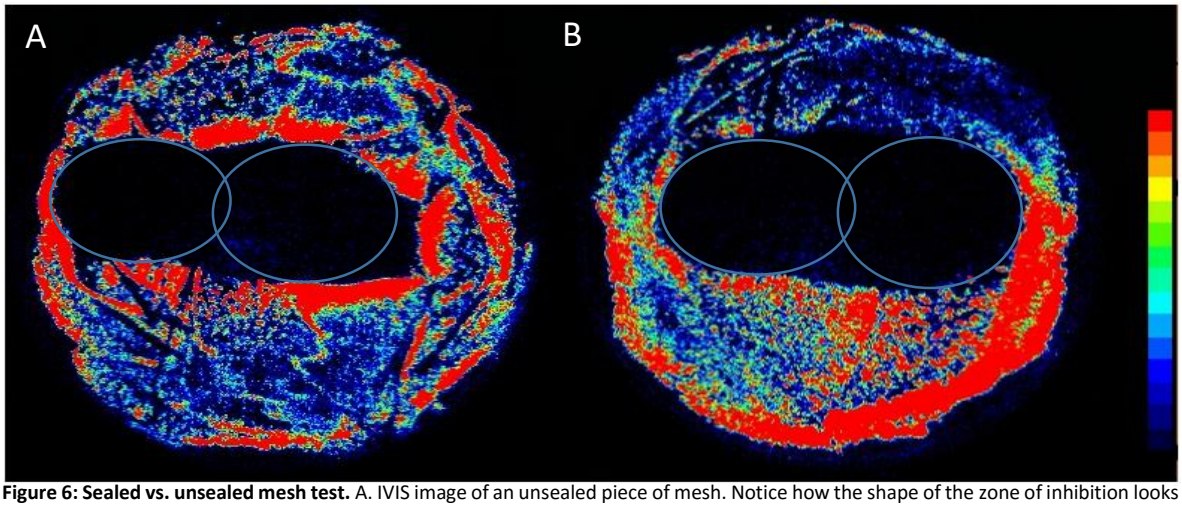

Figure 6: Sealed vs. unsealed mesh test. A. IVIS image of an unsealed piece of mesh. Notice how the shape of the zone of inhibition looks like two overlapping circles. This is because the antibiotic is leaking out of the ends of the mesh. B. IVIS image of a sealed piece of mesh. Notice the uniform shape of the zone of inhibition suggests that the antibiotic is eluting through the walls of the mesh.

DMSO charged disk, this could be due to the rifampicin solution mixing with the DMSO and the rifampicin using the DMSO to spread. This may have diluted the rifampicin in the DMSO area, leading to less complete inhibition. This test is shown in Figure 5B. The various DMSO tests gave varied results. Because each test was only repeated once, more replicates may help to clear up the confusion caused by the different tests. A sealed and an unsealed mesh were run against each other to determine whether the inhibitory effect was due to leakage out of the end or elution through the sides of the mesh. The difference was subtle, but the unsealed mesh had a zone of inhibition that looked like two circles centered at the ends of the mesh while the sealed mesh had a very uniform zone of inhibition. This result is shown in Figure 6. A similar test using longer pieces of mesh might give a less subtle difference between the two that could increase the confidence that it is actually eluting through primarily the walls.

CONCLUSIONS

The mechanical tests showed that the tensile strength of the hollow tubes in the longitudinal direction was enough to be used in the repair of ventral hernia. The UTS in the longitudinal direction was significantly stronger than the commercial mesh that was tested, and comparable to the meshes tested by Pott et al. [7] The novel mesh was significantly stiffer than the commercial mesh that was tested and

those tested by Pott et al., which could decrease the mesh's effectiveness in ventral hernia repair due to stress concentration in the surrounding tissue. [7] *In vivo* testing would need to be done to ensure that fibrosis does not occur around the surgical mesh. The pronounced anisotropy of the mesh is a cause for concern moving forward. Being a tenth as strong in the transverse direction may decrease its effectiveness in preventing recurrence of the ventral hernia. Possible ways to address this is to use a double-layered mesh, where the layers are perpendicular to each other, such that the transverse direction of one layer is aligned with the longitudinal direction of the other direction. Another way to deal with the anisotropy of the mesh might be to weave the hollow tubes of the mesh. Weaving or knitting the mesh together would help distribute the forces across the hollow tubes instead of the smaller fibers that connect the tubes. It would also change the highly parallel arrangement of the fibers to something much less parallel, which could have the effect of lowering the stiffness as well.

The antibiotic solution effectively prevented the growth of SAP-231, and it was likely not due to the DMSO used as a solvent. The DMSO helped with the solubility of rifampicin as well as with loading the mesh. DMSO has been approved by the FDA for one use, but its use has remained controversial despite minor and occasional side effects. [10] DMSO is well known for increasing the permeability of small molecules through membranes, so it would be beneficial to test the DMSO and rifampicin solution against a primary human cell culture to ensure that it inhibits the SAP-231 without inhibiting human cells. [10]

The hollow tube surgical mesh was an effective delivery tool for the antibiotic solution. There was an obvious zone of inhibition around the mesh, and tests suggest that it is due to elution through the walls of the mesh rather than leakage out of the ends. This is important for prevention of biofilms in ventral hernia repair because biofilms are known to form at the points where fibers cross over each other, and in a relatively long mesh, like ones used in ventral hernia repair, leakage out of the end will likely have less coverage at these points than elution through the walls. [5]

The use of a hollow tube surgical mesh in ventral hernia repair for the prevention of biofilms is feasible. The mesh can be made to elute an antibiotic that effectively inhibits the growth of *S. aureus*. More testing needs to be done to ensure there are no negative effects to primary human cells by the mesh, solvent, and antibiotic solution. The hollow tubes are sufficiently strong and stiff to be used in ventral hernia repair, but the mesh is too anisotropic to be used in its current form. There are also concerns over the mesh's stiffness, and the stress concentrations that occur because of it should be studied as the mesh is further developed.

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APPENDIX A: PICTURES

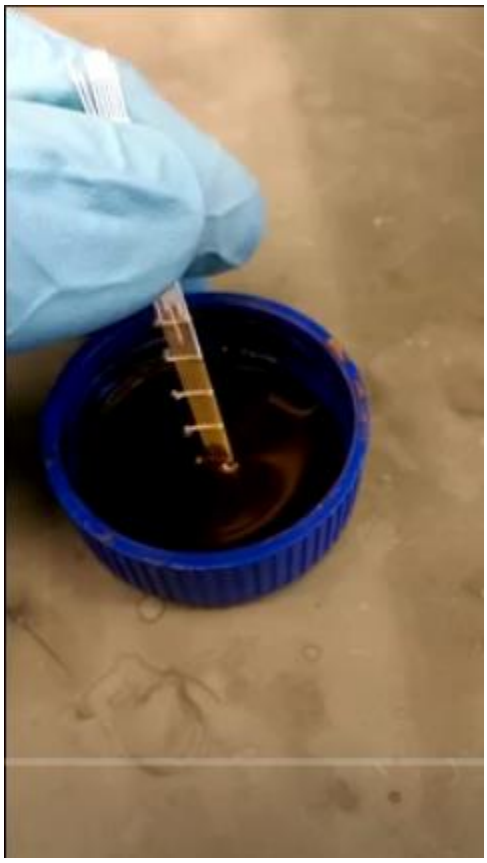

Figure A1: Filling the mesh with the rifampicin solution by taking advantage of the capillary effect.

Figure A2: The TESTRESOURCES testing frame that was used showing a typical sample break.

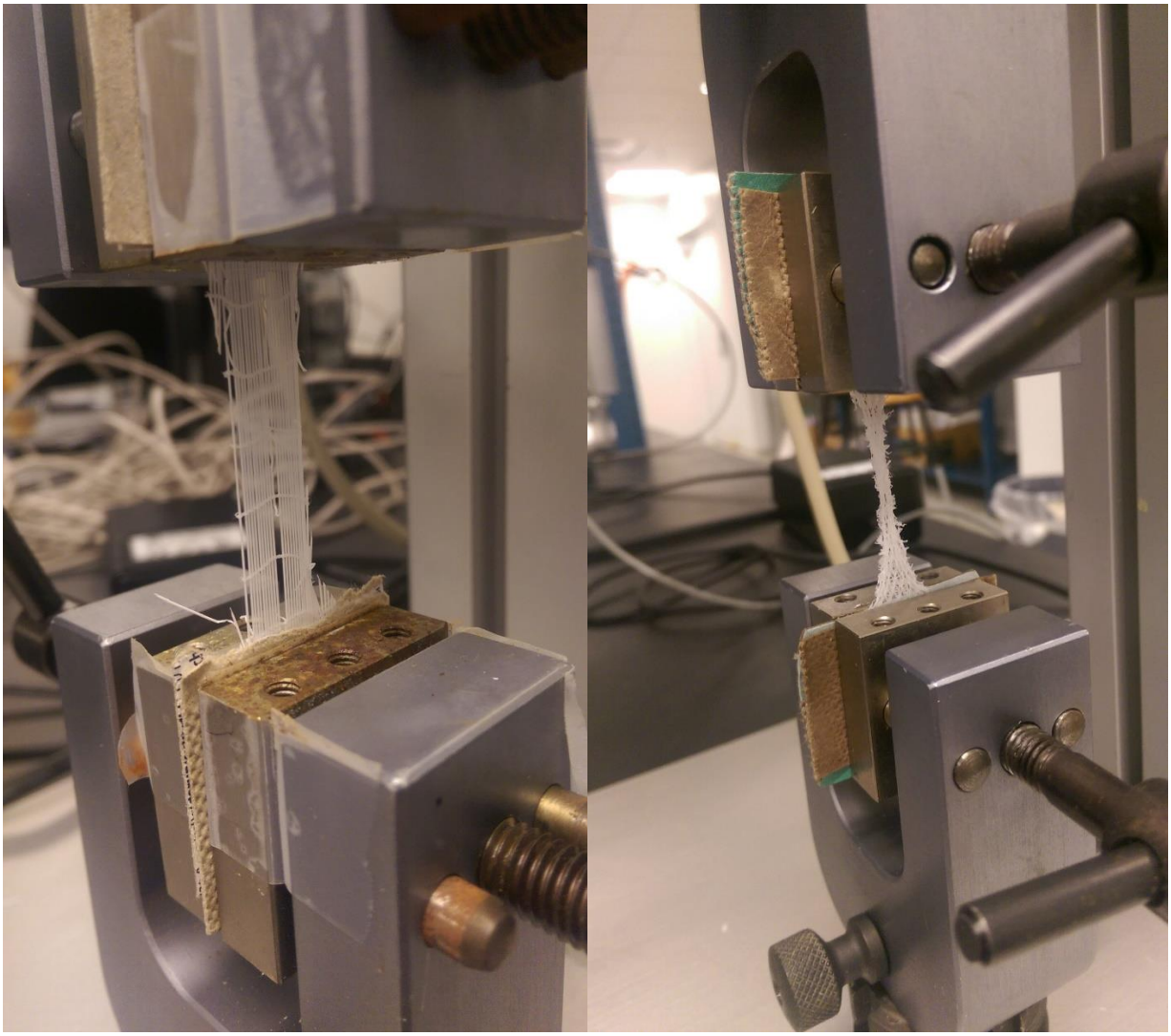

Figure A3: Close ups of sample breaks for the novel hollow tube mesh (left) and the commercial mesh (right).